IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: GILBERT and WAKARCHUK Application No.: 10/735,419 Filed: December 11, 2003 For: NUCLEIC ACIDS ENCODING SIALYTRANSFERASES FROM C. JEJUNI Customer No.: 20350	Confirmation No. 2617 Examiner: Sheridan Swope Technology Center/Art Unit: 1652 DECLARATION OF DR. MICHEL GILBERT UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michel Gilbert, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I, Dr. Gilbert, am currently Senior Research Officer at the Institute for Biological Sciences in the Glycosyltransferases and Neuroglycomics Group of the Glycobiology Program at the National Research Council of Canada (NRC). I received my B.Sc. in Microbiology from the Université Laval, Département de biochimie et microbiologie in Québec, Canada in 1986. I received my Ph.D. in Biology (biochemistry specialization) from the University of Ottawa, Department of Biology in 1992. I was a post doctoral fellow in the Laboratory of Dr. Rolf Morosoli at the Institut Armand-Frappier of Laval, Québec from 1993-1994. I joined the NRC in 1995 as a research associate in the laboratory of Dr. Warren Wakarchuk at the NRC laboratories in Ottawa as part of the Immunochemistry Program. In

1999, I was appointed Associate Research Officer of the NRC. In 2004, I was appointed Senior Research Officer of the NRC, the position I hold today. A copy of my curriculum vitae is attached hereto as Exhibit A.

3. The present invention is isolated or recombinantly produced sialyltransferase polypeptides that have α2,3 sialyltransferase activity, and that include an amino acid sequence that is at least 95% identical to SEQ ID NO:10.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of this patent application. In addition, I have read a preliminary Office Action, faxed and e-mailed to Beth Kelly on September 20, 2005, received in the present case. I have also read a reference included with the preliminary Office Action, Eichler *et al.*, *Carbohydrate Res.* 319:1-16 (1999). It is my understanding that the Examiner is concerned that the reference could support a rejection of the claims under 35 U.S.C. §102(a).

5. This declaration is provided to demonstrate that Eichler *et al.* does not anticipate the claimed invention. In particular, Eichler *et al.* does not disclose a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:10.

6. Eichler *et al.* disclose enzymatic sialylation of a pentasaccharide using the Cst-I sialyltransferase from *C. jejuni*. As disclosed in the specification at page 60, lines 12-14, Cst-II from *C. jejuni* strain NCTC 11168 (SEQ ID NO:10) shares only 44% identity with the first 300 amino acids of Cst-I, *i.e.*, the active site of the Cst-I. Eichler *et al.* does not disclose a protein with sialyltransferase activity that is at least 95% identical to SEQ ID NO:10.

7. In view of the foregoing, it is my scientific opinion that Eichler *et al.* does not disclose a protein with sialyltransferase activity that is at least 95% identical to SEQ ID NO:10. The reference, therefore, does not anticipate the claimed invention.

Date: 23 September 2005 By: Michel Gilbert

Michel Gilbert, Ph.D.

60583698 v1

MICHEL GILBERT

101 Sacré-Cœur, Apt. 116
Hull, Québec
Canada, J8X 1C7

Home : (819) 777-7687
Work : (613) 991-9956
email: michel.gilbert@nrc.ca

Research Interests:

I am interested in studying glycoconjugate biosynthesis in microbial systems. The fundamental aspects include identifying the enzymes and the mechanisms that are involved in the synthesis of lipopolysaccharides, polysaccharides and glycoproteins. This knowledge will help to understand better the roles of glycoconjugates in pathogenesis and can also result in the development of carbohydrate-based therapeutic agents and vaccines.

Education:

1993-1994 Postdoctoral position: Institut Armand-Frappier (Laval, Québec) Programme de microbiologie appliquée, Supervisor : Dr. Rolf Morosoli. Topic : Study of the secretion system of *Streptomyces lividans*.

1986-1992 Ph.D. in Biology (biochemistry specialization): University of Ottawa, Department of Biology, Supervisor: Dr. J.N. Saddler. Title of the thesis: "Production and characterization of the cellulases and xylanases from the thermophilic ascomycete *Thielavia terrestris* 255B".

1983-1986 B.Sc. in Microbiology: Université Laval, Département de biochimie et microbiologie, Québec.

Experience:

2004-now: Senior Research officer : Institute for Biological Sciences, Glycobiology Program, Glycosyltransferases and Neuroglycomics Group. National Research Council of Canada.

1999-2004: Associate Research officer : Institute for Biological Sciences, Immunochemistry Program, Pathogen Genomics Group. National Research Council of Canada.

-Supervisory experience: full-time supervision of a technical officer, a postdoctoral fellowship and of many summer and coop students. Co-supervision of a research associate and of many technical officers.

-Contributed to the writing of the research proposal for the "Genomics of Human Mucosal Pathogens" initiative.

EXHIBIT A

-Directed the work on the comparative genomics studies of the lipooligosaccharide biosynthesis locus from *Campylopbacter jejuni*.

-Supervised the sequencing of various carbohydrate biosynthesis loci in *Campylobacter jejuni* and used various bio-informatics tools to analyse the sequences.

-Directed a study on the association between specific *Campylobacter jejuni* glycosyltransferases and the development of Guillain-Barré and Miller-Fisher syndromes (in collaboration with Dr. Hubert Endtz, Erasmus University Medical Centre, Rotterdam, The Netherlands).

-Structure-function studies of various bacterial glycosyltransferases.

-Contributed to the development of chemi-enzymatic syntheses of carbohydrates under investigation in the carbohydrate-based vaccine program as part of a collaboration with Drs. Dennis Whitfield and Wei Zou.

-Collaboration with an industrial partner (Neose Technologies, Horsham, Pennsylvania): topic: development of glycosyltransferases for the synthesis of gangliosides and other glycoconjugates. Proposal writing, supervision of research activities, report writing and visits to their research facilities to present results and plan future work.

-Contributed to the writing of 5 patent applications (see below) in keeping with NRC strategy to insure proper protection of discoveries and technology developments resulting from research activities (intellectual property issues).

1995-1998: Research associate: Institute for Biological Sciences, Immunochemistry Program, Novel Antibodies and Proteins, National Research Council of Canada. Supervisor: Dr. W.W. Wakarchuk.

-Cloning of genes encoding bacterial glycosyltransferases that can be used for the synthesis of oligosaccharides with potential use as therapeutics. The cloning was performed using activity screening and genome analysis.

-Optimization of enzyme production by genetic engineering and optimization of fermentation conditions.

-Enzyme purification using chromatography techniques, ultrafiltration and precipitation techniques.

-Characterization of the acceptor specificity of the recombinant enzymes.

-Enzymatic synthesis of oligosaccharides with potential for use as therapeutics.

-Liquid and solid cultures of bacterial pathogens (BSL 2) such as *Neisseria meningitidis*, *Campylobacter jejuni* and pathogenic *Escherichia coli*.

-Collaboration with an industrial partner (Cytel Corporation, Glycotechnology division, San Diego, California): development of glycosyltransferases for the synthesis of oligosaccharides with biological activities. I performed experiments, supervised research activities, wrote reports and presented our results at their research facilities. Development of a fusion enzyme that was used to synthesize kg quantities of siallylactose.

1993-1994: Postdoctoral position: Institut Armand-Frappier, Département de microbiologie appliquée. Superviseur : Dr. Rolf Morosoli. Topic : Study of the secretion system of *Streptomyces lividans*:

-Cloning, sequencing and characterization of genes encoding secretion factors.

-Production of recombinant proteins by prokaryotes (*Escherichia coli* and *Streptomyces lividans*).

-Site-directed mutagenesis.

1986-1992: Ph.D. thesis research (University of Ottawa, Department of Biology): the research was carried out at the Eastern Laboratory of Forintek Canada Corp.:

-Study of fungal enzymes (carbohydrate-degrading enzymes such as xylanases and cellulases) with potential industrial applications.

-Production and purification of enzymes.

-Enzyme characterization: physico-chemical properties and kinetics.

-Study of enzyme mechanisms and enzyme-enzyme interactions.

Professional Associations:

Member of the "Canadian Society of Microbiologists"

Patents:

Gilbert, M. and Wakarchuk, W.W. Nucleic acids encoding β-1,4-GalNAc transferase. U.S. Patent No. 6,911,337 (Issued on June 28, 2005).

Gilbert, M. and Wakarchuk, W.W. Nucleic acids encoding polypeptides with β1,3-galactosyl transferase activity. U.S. Patent No. 6,905,867 (Issued on June 14, 2005).

Gilbert, M. and Wakarchuk, W.W. Polypeptides having β-1,3-Galactosyl transferase activity. U.S. Patent No. 6,825,019 B2 (Issued on November 30, 2004).

Gilbert, M. and Wakarchuk, W.W. Polypeptides having β-1,4-GalNAc transferase activity. U.S. Patent No. 6,723,545 (Issued on April 20, 2004).

Gilbert, M. and Wakarchuk, W.W. Lipopolysaccharide α-2,3-sialyltransferase of *Campylobacter jejuni* and its uses. U.S. Patent No. 6,689,604 (Issued on February 10, 2004) and US Patent No. 6,709,834 (Issued on March 23, 2004). Australian Patent No. 745040 (issued on June 20, 2002).

Gilbert, M., Young, N.M. and Wakarchuk, W.W. Fusion proteins for use in enzymatic synthesis of oligosaccharides. Australian Patent No. 754926 (Issued on March 13, 2003).

Gilbert, M. and Wakarchuk, W.W. *Campylobacter* glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics. U.S. Patent No. 6,503,744 (Issued on January 1, 2003) and US Patent No. 6,699,705 (Issued on March 2, 2004). Australian Patent No. 772569 (Issued on August 12, 2004).

Gilbert, M., Wakarchuk, W.W., Young, N.M., Jennings, M.P. and Moxon, E.R. Recombinant α-2,3-sialyltransferases and their uses. U.S. Patent No. 6,210,933 (issued on April 3, 2001) and U.S. Patent No. 6,096,529 (issued on August 1, 2000). European Patent No. 0906432 (issued on June 9, 2004).

Recent publications:

Li, J., Koga, M., Brochu, D., Yuki, N., Chan, K. and **Gilbert, M.** 2005. Electrophoresis-assisted open-tubular liquid chromatography / mass spectrometry for the analysis of lipooligosaccharide expressed by *Campylobacter jejuni*. *Electrophoresis* **26**:3360-3368.

Blixt, O., Vasiliu, D., Allin, K., Jacobsen, N., Warnock, D., Razi, N., Paulson, J.C., Bernatchez, S., **Gilbert, M.** and Wakarchuk W.W. 2005. Chemoenzymatic Synthesis of 2-azidoethyl-Ganglio-oligosaccharides GD3, GT3, GM2, GD2, GT2, GM1 and GD1a. *Carbohyr. Res.* **340**:1963-1972.

Parker, C.T., Horn, S.T., **Gilbert, M.**, Miller, W.G., Woodward, D.L. and Mandrell, R.E. 2005. Comparison of *Campylobacter jejuni* lipooligosaccharide biosynthesis loci from a variety of sources. *J. Clin. Microbiol.* **43**:2771-2781.

Koga, M., **Gilbert, M.**, Li, J., Koike, S., Takahashi, M., Furukawa, K., Hirata, K. and Yuki, N. 2005. Antecedent infections in Fisher syndrome: a common pathogenesis of molecular mimicry. *Neurology* **64**:1605-1611.

Gunawan, J., Simard, D., **Gilbert, M.**, Lovering, A.L., Wakarchuk, W.W., Tanner, M.E. and Strynadka, N.C. 2005. Structural and mechanistic analysis of sialic acid synthase NeuB from *Neisseria meningitidis* in complex with Mn^{2+}, phosphoenolpyruvate, and *N*-acetylmannosaminitol. *J. Biol.Chem.* **280**:3555-3563.

Karlyshev, A.V., Champion, O.L., Churcher, C., Brisson, J.-R., Jarrell, H.C., **Gilbert, M.**, Brochu, D., St. Michael, F., Li, J., Wakarchuk, W.W., Goodhead, I., Sanders, M., Stevens, K., White, B., Parkhill, J., Wren, B.W. and Szymanski C.M. 2005. Analysis of *Campylobacter jejuni* capsular loci reveals multiple mechanisms for the generation of structural diversity and the ability to form complex heptoses. *Mol. Microbiol.* **55**:90-103.

Godschalk, P.C.R., Heikema, A.P., **Gilbert, M.**, Komagamine, T., Ang, C.W., Glerum, J., Brochu, D., Li, J., Yuki, N., Jacobs, B.C., van Belkum, A. and Endtz, H.P. 2004. The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barré syndrome. *J. Clin. Invest.* **114**:1659-1665.

Zou, W., Borrelli, S., **Gilbert, M.**, Liu, T., Pon, R.A. and Jennings, H.J. 2004. Bioengineering of surface GD3 ganglioside for immunotargeting human melanoma cells. *J. Biol.Chem.* **279**: 25390-25399.

Chiu, C.P.C., Watt, A.G., Lairson, L.L., **Gilbert, M.**, Lim, D., Wakarchuk, W.W., Withers, S.G. and Strynadka, N.C.J. 2004. Structural analysis of the sialyltransferase Cst-II from *Campylobacter jejuni* in complex with a substrate analog. *Nature Struc. Mol. Biol.* **11**:163-170.

Gilbert, M., Godschalk, P.C.R., Karwaski, M.-F., Ang, C.W., van Belkum, A., Li, J., Wakarchuk, W.W. and Endtz, H.P. 2004. Evidence for the acquisition of the lipooligosaccharide biosynthesis locus in *Campylobacter jejuni* GB11, a strain isolated from a Guillain-Barré syndrome patient, by horizontal exchange. *Infec. Immun.* **72**:1162-1165.

Szymanski, C.M., St.Michael, F., Jarrell, H.C., Li, J., **Gilbert, M.**, Larocque, S., Vinogradov, E. and Brisson, J.-R. 2003. Detection of conserved *N*-linked glycans and phase-variable lipooligosaccharides and capsules from *Campylobacter* cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. *J. Biol. Chem.* **278**:24509-24520..

Antoine, T., Priem, B., Heyraud, A., Greffe, L., **Gilbert, M.**, Wakarchuk, W.W., Lam, J.S. and Samain, E. 2003. Large-scale *in vivo* synthesis of the carbohydrate moieties of gangliosides GM1 and GM2 by metabolically engineered *Escherichia coli*. *ChemBioChem* 4:406-412.

Yan, F., Mehta, S., Eichler, E., Wakarchuk, W.W., **Gilbert, M.**, Schur, M.J. and Whitfield, D.M. 2003. Simplifying oligosaccharide synthesis: efficient synthesis of lactosamine and sialylated lactosamine oligosaccharide donors. *J. Org. Chem.* **68**:2426-2431.

Karwaski, M.-F., Wakarchuk, W.W. and **Gilbert, M.** 2002. High-level expression of recombinant *Neisseria* CMP-sialic acid synthetase in *Escherichia coli*. *Prot. Expr. Purif.* **25**:237-240.

Priem, B., **Gilbert, M.**, Wakarchuk, W.W., Heyraud, A. and Samain, E. 2002. A new fermentation process allows large-scale production of human milk oligosaccharides by metabolically engineered bacteria. *Glycobiology* **12**:235-240.

Tremblay, D., Lemay, J., **Gilbert, M.**, Chapdelaine, Y., Dupont, C. and Morosoli, R. 2002. High-level heterologous expression and secretion in *Streptomyces lividans* of two major antigenic proteins from *Mycobacterium tuberculosis*. *Can. J. Microbiol.* **48**:43-48.

Gilbert, M., Karwaski, M.-F., Bernatchez, S., Young, N.M., Taboada, E., Michniewicz, J., Cunningham, A.-M. and Wakarchuk, W.W. 2002. The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, *Campylobacter jejuni*: biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. *J. Biol. Chem.* **277**:327-337.

Yan, F., **Gilbert, M.**, Wakarchuk, W.W., Brisson, J.-R. and Whitfield, D.M. 2001. Chemoenzymatic iterative synthesis of difficult linkages of oligosaccharides on soluble polymeric supports. *Organic Lett.* **3**:3265-3268.

van Belkum, A., van den Braak, N., Godschalk, P., Ang, W., Jacobs, B., **Gilbert, M.**, Wakarchuk, W., Verbrugh, H. and Endtz, H. 2001. A *Campylobacter jejuni* gene associated with immune-mediated neuropathy. *Nature Med.* **7**:752-753.

Wakarchuk, W.W., Watson, D., St.Michael, F., Li, J., Wu, Y., Brisson, J.-R., Young, N.M, and **Gilbert, M.** 2001. Dependence of the bi-functional nature of a sialyltransferase from *Neisseria meningitidis* on a single amino acid substitution. *J. Biol. Chem.* **276**:12785-12790.

Mosimann, S.C., **Gilbert, M.**, Dombrowski, D., To, R., Wakarchuk, W. and Strynadka, N.C.J. 2001. Structure of a sialic acid-activating synthetase, CMP-acylneuraminate synthetase in the presence and absence of CDP. *J. Biol. Chem.* **276**:8190-8196.

Hood, D.W., Cox, A.D., **Gilbert, M.**, Makepeace, K., Walsh, S., Deadman, M.E., Cody, A., Martin, A., Mansson, M., Schweda, E.K.H., Brisson, J.-R., Richards, J.C., Moxon, E.R. and Wakarchuk, W.W. 2001. Identification of a lipopolysaccharide α-2,3-sialyltransferase from *Haemophilus influenzae*. *Mol. Microbiol.* **39**:341-350.

Book chapters:

Gilbert, M., Godschalk, P.C.R., Parker, C.T., Endtz, H.P. and Wakarchuk, W.W. 2005. Genetic bases for the variation in the lipooligosaccharide outer core of *Campylobacter jejuni* and possible association of glycosyltransferase genes with post-infectious neuropathies. In *Campylobacter: Molecular and Cellular Biology*. (Horizon Bioscience; Editors: J.M. Ketley and M.E. Konkel), Chapter 11, pp. 219-248.

Thibault, P., Martin, A., **Gilbert, M.**, Wakarchuk, W.W. and Richards, J.C. 2003. Analysis of bacterial glycolipids by capillary electrophoresis-electrospray mass spectrometry. In *Methods in Molecular Biology*, Vol. 213: Capillary Electropheresis of Carbohydrates (Humana Press Inc., Editors: P. Thibault and S. Honda), pp. 241-259.